

Shutting Down Genes in Cancer, Bacteria, and Viruses

RIBONUCLEIC acid (RNA) interference is a relatively new technique in which small molecules called short interfering RNA (siRNA) can be inserted into cells to turn off a chosen gene. The potential uses for this technique in the fields of functional genomics and drug discovery are far reaching. In fact, because RNA interference can suppress tumor-causing genes, it may even lead to a cure for cancer.

However, several barriers have stymied attempts to apply siRNA techniques to research and clinical use. Current siRNA molecules cannot function in bacteria, which are widely used in biological research experiments. The siRNA molecules also cannot be delivered into cells without disrupting the cells, and the genes do not remain suppressed for long.

Scientists in Livermore's Biology and Biotechnology Research Program Directorate have overcome the problems of delivery and the duration of effectiveness by changing the molecular composition of the conventional double-stranded RNA molecules used in siRNA. In research funded by the Laboratory Directed Research and Development Program, they combined a single strand of RNA with a complementary single strand of DNA to create short hybrid RNA-DNA molecules, called siHybrids. The project team, which is led by biomedical scientist Allen Christian, received a 2004 R&D 100 Award for the novel technology.

The siHybrids are inherently more stable than siRNAs. They passively enter cells through a mechanism not yet understood by the Livermore researchers and remain stable in the presence of the enzymes in a cell. As a result, the hybrid molecules have a more robust efficiency than siRNA, and their effects last up to 10 times longer than those of the conventional molecules. Additionally, siHybrids cost half as much to produce, and unlike siRNA, they are effective in bacteria.

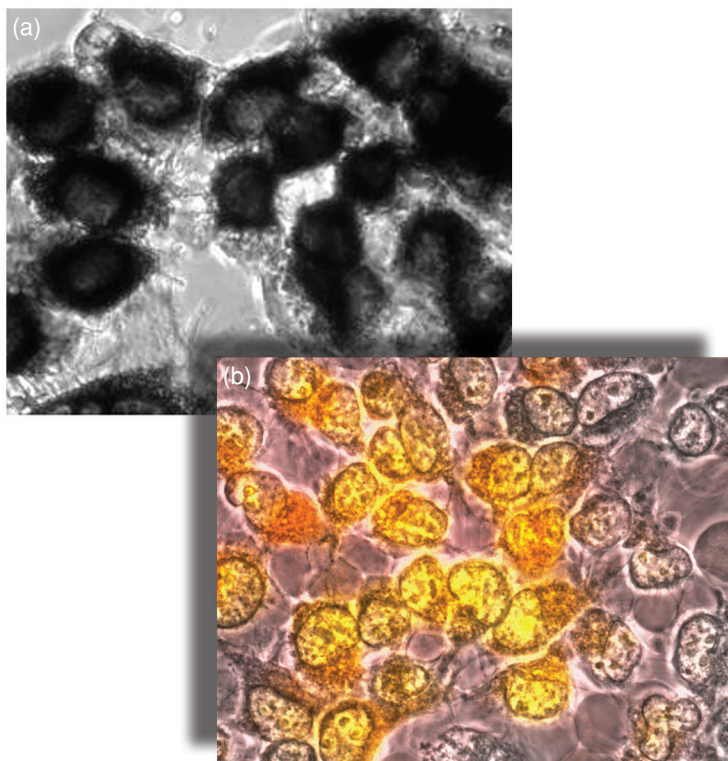
"With the siHybrids, researchers and physicians could quickly, inexpensively, and precisely shut off a damaged or abnormal gene that is causing a disease, ranging from cancer to bacterial or viral infection," says Christian. A single cell contains more than 6 billion nucleotide bases. Yet, siHybrids can locate the one damaged or misplaced gene in a cell and quickly render it quiescent.

How siHybrids Work

Genes act on the body through the expression of the specific proteins and enzymes that each gene encodes. The genetic code is stored in the DNA sequence, which is transcribed into RNA and finally translated into a polypeptide—that is, proteins, enzymes, or peptide hormones. Genetic disorders result from damage to a gene, which in turn manufactures an abnormal gene product.

Gene silencing by RNA interference attempts to keep detrimental proteins from harming the body by suppressing their expression. These intervention strategies are not limited to silencing endogenous genes—those that occur naturally in the body. They can also be used to silence foreign genes, such as those from an invading virus or bacteria, thereby protecting the body from infection.

To use siHybrids, a technician first identifies a sequence of nucleotides that make up the target gene. Then a piece of RNA-DNA hybrid about 20 nucleotides long is synthesized to match



Livermore's gene-silencing technology, called siHybrids, has been used successfully to suppress cancer-causing genes. Here, micrographs show cancer cells (a) before and (b) after treatment with siHybrids.

part of the identified genetic sequence. When this inhibiting hybrid molecule is inserted into a cell, it selectively degrades the messenger RNA of the target sequence, which disables the gene's capacity to manufacture its product.

According to Christian, when the Livermore team used siHybrid molecules to target out-of-control cancer genes, experimental results showed that the molecules stopped the rapid growth of cancerous cells. Even more promising is the fact that the treatment had no deleterious effect on the normal cells surrounding the cancer.

A Potential Cancer Therapy

Now that the sequencing of the human genome has been completed, scientists working in functional genomics have turned their attention to identifying the function of each gene and developing corresponding mechanisms to treat genetic disorders. The siHybrid molecules are an effective tool for this research area, providing researchers with a fast, automated test of gene function.

The hybrid molecules are particularly advantageous for the diagnosis and treatment of cancer. Traditional RNA interference applications, including siRNA, require special delivery systems to transport the molecules into the cells. But many delivery agents are toxic to the body, and existing delivery methods are too harsh for use in humans. However, siHybrids enter cells freely without a delivery system. In addition, they are more effective than present RNA interference technologies. Because genes remain suppressed longer when cells are treated with siHybrids than with siRNA, the hybrid molecules are ideal for long-term clinical applications.

With these advantages, siHybrids have the potential to dramatically improve cancer therapy by directly shutting down the cancer-causing genes. Studies indicate that cancer occurs when the expression of mutated genes or the overexpression of native genes stimulates uncontrolled cell division. The Livermore team has demonstrated that siHybrids will silence such genes in cells. For example, adding siHybrids to cell-growth media selectively shuts down the gene that causes prostate cancer. In the laboratory, they also effectively silenced the mutated tumor-suppressor gene that is suspected of being involved in 50 to 55 percent of all human cancers. Other possible cancer targets for siHybrids include suppressing genes that encode drug-resistant proteins, growth factors, and receptors in specific tumors.

From Genetic Disorders to Infectious Diseases

Christian foresees that siHybrid technology could have numerous applications. For example, virtually all genetic disorders—from neonatal and neurogenerative diseases to immunodeficiencies—are caused by mutated gene expression, which could be disrupted or diminished with siHybrid treatment. This technology also may be effective against bacterial infection, plus the treated organism will not develop a resistance to the siHybrids. The hybrid molecules may even prove successful

in shutting down viruses that have already infected a host. In addition, says Christian, applications are possible in such areas as agriculture, toxicology, pharmacology, immunology, veterinary medicine, embryology, environmental science, and counterterrorism.

—Maurina S. Sherman

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Members of the siHybrids team: (standing, from left) Alice Chen, Allen Christian, and Erik Hofmann; (seated) Larry Dugan. Not pictured: Janelle Lamberton and Rose Latham.